A TAXONOMIC STUDY OF THE GENUS LAURENCIA (CERAMIALES, RHODOPHYTA) FROM VIETNAM. III. LAURENCIA CALLICLADA SP. NOV.

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ABSTRACT — Luneronia callicitatis Massuda sp. nov. (Ceramiales, Rhodophyta), described from Vietnam, is chantectived by the following set of morphological features: 1) a hasal system composed of a single disc from which a few, small, slender, softly flestly, terete, upright axes develop; 2) the production of four periaxial cells from each axial cell; 3) the presence of longitudinally oriented scondary pri-connection between contiguous susperifical cortical cells; 4) surface cortical cells that project in the upper portion of branches; 5) an absence of lenticular thickenings in the walls of medullary cells; and 6) a parallel arrangement of tetrasporangia. Furthermore, in produces a triterpenoid, calliciadoi, as a major halogenated secondary metabolite that is unknown in any other agala species.

RESUMÉ Laurencia culticiaia Masuda sp. nov. (Ceramiales, Rhodophyta), espèce decrite du Victoram, est caractérisée par l'ensemble des particularies morphologiques suivantes; 1) un système basai composé d'un disque d'où se développent quelques petits axes dresses ténus, mollement charmis, de section arrondie; 2) le production de quatre cellules périssiales à partir de chaque cellule sainle; 3) la présence de symapus secondaires orientées longitudinalement, entre les cellules corticales superficielles contigués; 4) des cellules corticales fisants saillé a la surface des parties apicales des rameaux; 5) l'absence d'éspaississements lenticulaires dans la paroi des cellules médullaires; cl 6) une disposition parallèle des étrasporcystes. En outre, cette espèce produit, comme principal métabolite secondaire halogèné, un triterpénoïde, le callicladol, qui n'est présent dans aucune autre espèce d'altrue. l'Fradult par la Rédaction, le callicladol, qui n'est présent dans aucune autre espèce d'altrue. l'Fradult par la Rédaction le

KEY WORDS: Ceramiales, chemotaxonomy, halogenated secondary metabolite, Laurencia cullicluda, Rhodophyta, algae.

INTRODUCTION

Along Vietnamese coasts 21 species of the red algal genus Laurencia (Rhodomelaceae, Ceramiales) have been reported (Dawson, 1954; Pham, 1969; Nguyen et al., 1993; Masudae et al. 1997a, b.c, d). However, some species, which require further studies to confirm their status, may be included, as Cribb (1983) suggested that L. paniculata (C. Agardh). Pagardh reported by Dawson (1954) is referable to L. concreta Cribb. This number is smaller than that of Chinese coasts where 30 species have been recorded (Zhang & Xia, 1988). It has been expected that more species would be found in Vietnamese waters. Our Vietnamese expedition during 1992 and 1993 reveals that many undescribed or unrecorded species of Laurencia are present (Suzuki et al., 1995, 1996; Masuda et al., unpublished observations).

It was reported in an earlier paper (Suzuki et al., 1995) that a new triterpenoid, callicladol, had been extracted from an undescribed species, Laurencia calliclada Masuda sp. ined. The formal description of this species is now given.

MATERIALS AND METHODS

Specimens were collected on 8 February 1993 at An Thoi, Phu Quac Island, Kien Giang Province, Vetram, and were fixed in 4% formalin in seawater. Some were dried as voucher herbarium of the Graduate School of Science, Hokkaido University (SAP 06.092-06.095). Sections were made by hand using a razor folade and pith stack. These were standed with 0.5% (w/v) cotton blue in a lateful acid/phenol/glycerol/water (1:11:1) solution and mounted in 50% glycerol-seawater on microscope slides.

OBSERVATIONS

Laurencia calliclada Masuda, sp. nov.

Plantae axibus rectis pluribus e disco basali communi effecti. flavida-rubra vel scarlatina. carnosa, mollis, exsiccatione chartue adhaverntes: thaili 2-4 cm alti. teretes. axibus principalibus percurrentibus: axes principales usugu ed 1200 um in diamiento, ramos numerosos in mode tregulariter spirali ferentes; cellula axialis omnis cum cellulas periaxialibus quantum cellulae ostaleae superficieres e tiem prope apices ramorum non procurrentes, cum foveis-colligationibus secundariis longitudinaliter dispositis inter se, in sectionibus transversalibus ramuli nor endatiam elongatus ne cei in sallem dispositae; inerascationes lenticulares in parietibus cellularum medullae absentes; tetrasporangia e cellulai periaxialibus in romis ultimis et penulimis in successione acropteali formata, igitur in ordinatione parallela ad axem longitudinalment medisposita, tetrasporangia matura 100-120 mm indunetro, cysto

carpia laterales in ramis, ovoidea, 600-800 µm alta, 500-740 µm lata; spermatangia non invasta

Plants with several upright axes arising from a common discoid holdfast, yellowish-red or scarlet, fleshy, soft, adhering to paper on drying; thali? 4c m high, teret throughout, with percurrent main axes; main axes up to 1200 µm in diameter, bearing may branches in an irregularly spiral manner; each axial cell with four periaxial cells, superficial cortical cells projecting at the upper portion of branches, with longitudinally oriented secondary pit-connections between them, in transverse sections of branchlets neither clongated radially nor arranged as a palisade; lenticular thickenings absent in the walls of medullary cells; tetrasporangia formed from periaxial cells on ultimate and penultimate branches in aeropetal succession, therefore arranged in parallel to the longitudinal axis; mature tetrasporangia 100-120 µm in diameter; cystocarps lateral on branches, ovoid, 600-800 µm high, 500-740 µm wide; spermatangia not found.

Holotype and type locality: SAP 062095 (tetrasporangial specimen, Fig. 1), collected at An Thoi, Phu Quac Island, Kien Giang Province, Vietnam (8.ii.1993) by M. Masuda

Distribution: Endemic; known only from the type locality, facing the Gulf of Thailand, southern Vietnam.

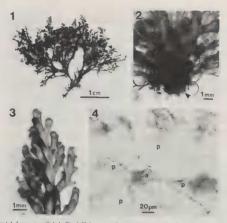
Etymology: The specific epithet is derived from two Greek compounds, calli-(beautiful) and -cladus (branch).

Plants grow on rocks or corallinaceous algae in the lower intertidal zone. Eight to twenty upright axes, including very small ones (detected under a dissecting microscope), arise from a common dissoid holdfast 2.5-3.5 mm in diameter [Fig. 2] and have no creeping branches which function as secondary attachment organs; only one to four upright axes, however, fully mature. Thalli are 2-4 cm high, yellowish-red or searlet, fleshy and soft, and definer firmly to paper when dried. Main axes are prerurent and terete, 600-900 µm in diameter just above the basal disc, 800-1200 µm in the lower portion, then tamer gradually to 500-700 µm distally.

Main axes bear many first-order branches in an irregularly spiral manner (Fig. 3) at intervals of 0.5-2.0 mm and at angles of 30-60°. First-order branches are 1.5-2.5 cm in length on lower to middle portions of the main axis and form progressively shorter branches of up to five orders. Branches of all orders show a polystichous arrangement. Adventitious branches are formed on main axes and lower portions of first-order branches.

The growing point is always sunk in an apical pit, as is typical of the genus. Axial cells are recognizable only near the apical cell, each producing four periaxial cells (Fig. 4). Superficial cortical cells of distal parts of branches of all orders are polygonal, 12-24 µm long by 16-32 µm wide (a length-width ratio of 0.5-1.0), and are regularly arranged in longitudinal rows in surface view. Superficial cortical cells of proximal parts of well-developed branches are 34-100 µm long by 26-50 µm wide (a length-width ratio of 10.3.3).

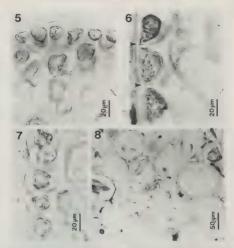
Superficial cortical cells in transverse section are 16-22 µm thick in upper portions of first-order branches and 36-60 µm thick in proximal portions. They are neither radially elongated nor form a palisade layer (Fig. 5). Longitudinally oriented secondary pit-connections are present between contiguous superficial cortical cells (Fig. 6). Superficial cortical cells project slightly in upper portions of Pranches (Fig. 7). Lenticular thickenings are not found in the walls of medullary cells (Fig. 8), which are up to 190 µm in diameter (walls being up to 2 µm thick) in lower portions of the first-order branches. As living material was not available, examination of corpse or cerise was not performed.



Figs 1-4. Laurencia colliclaulu. Fig. 1. Holotype specimen (tetrasporangial) collected at An Thoi. Phu Quae Island, Kien Giang Provine. Victnam (SAP 062095). Fig. 2. The basal disc (arrowhead) and numerous upright axes. Fig. 3. Uppermost portion of a teret, radially branching axis. Fig. 4. The four periaxial cells (p).

Tetrasporangia are formed on ultimate and penultimate, ordinary (Fig. 9) and adventitious branches that are 900-2000 µm long by 500-600 µm wide. The tetrasporangial initial is cut off from an elongated periaxial cell towards the abaxial side (Fig. 10). In each fertile segment, only two of the four periaxial cells elongate (Fig. 11) and produce tetrasporangia. Each tetrasporangiam is associated with two cover cells which are distally produced by the fertile periaxial cell (Fig. 12). Tetrasporangia mature acropetally, the young to almost mature sporangia showing a parallel arrangement along the fongitudinal axis of the branch (Fig. 9). Mature tetrasporangia are 100-120 µm in diameter.

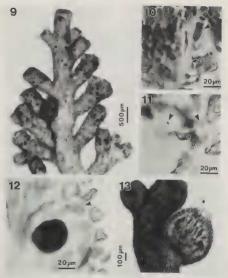
Cystocarps are borne laterally on branches of every order. Mature cystocarps are ovoid with a slightly beaked ostiole (Fig. 13) and 600-800 µm high by 500-740 µm wide. Spermatangia were not observed.



Figs 5.8. Laurencia calificatas. Fig. 5. TS of the distal portion of a third-order branch. Fig. 6. Longitudinal section(LS) of the lower portion of a first-order lateral showing longitudinally oriented secondary pit-connection (arrowheads) between contiguous superficial cortical cells. Fig. 7. LS of the distal portion of a third-order branch showing projecting superficial cortical cells. Fig. 8. TS of the lower portion of a first-order branch showing medullary cells lacking lenticular thickenings.

DISCUSSION

The following features have been used in combination to distinguish species in modern taxonomic treatments of the genus *Lauvencia* (Saito, 1967, 1969; Saito & Womersley, 1974; Cribb, 1983; McDermid, 1988; Zhang & Xia, 1988; Nam & Saito, 1990, 1992; Vandermeulen *et al.*, 1990; Wynne & Ballantine, 1991; Gil-Rodriguez & Haroun, 1992;



Figs 9.13. Laurencia cullicadus. Fig. 9. Upper portion of a second-order branch bearing tetrasporangial branchlets. Fig. 10. LS of a tetrasporangial branch showing a young tetrasporangium (arrowhead) borne on an elongated, fertile periaxual cell (arrow), Fig. 11. TS of a tetrasporangial branch showing an axial cell (a), one vegetative periaxual cell (p) (a second being out of focus) and two probable fertile periaxual cells (arrowheads). Fig. 12. LS of a tetrasporangial branch, a more developed tetrasporangium associated with one (arrowhead) of two cover cells. Fig. 13. Globular cystocarp with a slightly beaked ostiole.

Maggs & Hommersand, 1993; Masuda & Abe, 1993; Nam & Sohn, 1994; Ballantine & Aponte, 1995; Fujii & Cordeiro-Marino, 1996; Fujii et al., 1996; Masuda et al., 1996, 1997a, b, c, d); 1) the number (four or two) of vegetative periaxial cells per axial cell; 2) the hasal attachment system whether consisting of a single primary discoid holdfast only, or a single primary discoid holdfast plus secondary holdfasts formed on prostrate, stoton-like branches, a prostrate primary axis, or descending lower branches; 3) the presence or absence of percurrent main axes; 4) the shape of main axes in transverse section, whether terete to subterete or compressed to flattened, and the dimension; 5) the branching pattern, whether spiral (the arrangement being either polystichous or tristichous), distithous unitateral, verticillate or distichous-opposite; 6) the presence or absence of longitudinally oriented secondary pit-connection between contiguous superficial cortical cells; 7) the presence or absence of a surface-cortical palisade layer; 8) the presence or absence of projecting superficial cortical cells; 9) the presence or absence of lenticular thickenings in the walls of medullary cells; 10) the tetrasporangial arrangement relative to the longitudinal axis of the branchict, whether parallel or perpendicular, 11) the presence or absence of additional tetrasporangium-bearing periaxial cells; 12) the position of tetrasporangium-bearing periaxial cells; 13) the shape of cystocarps; 14) the presence or absence of short papilliform branchlets; 15) the presence or absence of hamate (hooked) branches; 16) the presence or absence of deciduous branchlets that function as propagules; 17) the presence, absence and number per cell of carps en cerise; 18) the presence or absence of iridescence; 19) the dimensions of superficial cortical cells; 20) the dimension of medullary cells; 21) the presence or absence of intercellular spaces between outer cortical cells; and 22) the presence or absence of an outermost translucent layer. In addition, plant size, texture (whether soft or rigid, fleshy or cartilaginous) and colour have been used. Of these, the basal attachment system should be considered of primary importance because many investigators have described it, and it is known to be highly invariant (Saito, 1967; Cribb, 1983; Maggs & Hommersand, 1993; Masuda & Abe, 1993). Species with a basal system similar to L. calliclada are relatively few. Those species that require comparison with the alga under study are as follows.

Several Australian and Asian Laurencia species posses a primary discoid holdfast only. Of these, several also have longitudinally oriented secondary ph-connections between contiguous superficial cortical cells as in L. cultivatud. Of these, five can be distinguished from L. cultivatud by the absence of projecting superficial cortical cells: L. clauvata Sonder (Saito & Womersley, 1974), L. flifformis (C. Agardh) Montagne (Saito & Womersley, 1974 as L. filiformis 1, filiformis), L. forsteri (Mertens ex Turner) Greville (Saito & Womersley, 1974), L. sphepherali Saito er Womersley (1974), and L. tropical Yamada (Masuda, unpublished observations). The presence or absence of projecting superficial cortical cells is an important specific feature (Yamada, 1931; Satto, 1969). Nan & Saito, 1990; Masuda & Abe, 1993), Laurencia filiformis and L. forsteri additionally differ from L. callicalain having lenticular thickenings (Saito & Womersley, 1974). The taxonomic usefulness of lenticular thickenings fast already been justified and discussed in detail (Masuda et al., 1992, 1966, and references cited therein).

Laurencia silvae Zhang et Xia (1980, as L. fasciculata Zhang et Xia), described on the basis of material from the Xisha Islands, China, is similar to L. calliclada in the majority of its features. However, L. silvae has lenticular thickenings, plus dense clusters of branchlets in an abbreviated alternate arrangement (Zhang & Xia, 1980), unlike L. calliclada. Tetrasporangial branches of L. silvae are mostly 300-650 µm long, whereas those of L. calliclada are 1900-2000 µm long.

Laurencia saitoi Perestenko (1980), described on the basis of material from Peter

the Great Bay, Russia, and known from the western Pacific (Masuda & Abe, 1993), also shares many critical features including the absence of lenticular thickenings with L calificlata (Masuda & Abe, 1993). The clearest difference between the two species is the number of upright axes in L. sation (Masuda & Abe, 1993). The clearest difference between the two species is the number of upright axes in L. sation (Masuda & Abe, 1993) versuse 3-20 in L. calificlatal. Furthermore, L. sation has larger (up to 16 cm long), purplish-brown to dark purplish-red thalli and thicker main axes (up to 2.5 mm wide) (Saito, 1967, as L. obtusa (Huston) Lamouroux; Masuda & Abe, 1993). These two species also produce quite different halogenated secondary metabolites, L. calificlada producing only the triterpenoid callicladio whereas L. sation producing only the triterpenoid callicladio whereas L. sation iproducins and several triterpenoids as major compounds (Takeda et al., 1990, as L. obtusa; M. 1985, 1987, as L. obtusa; The taxonomic importance of halogenated metabolites has been defended and discussed in detail by Masuda et al. (1996).

Laurencia suitor is mainly distributed along warm temperate coasts in Japanese and adjacent waters (Masuda & Abe, 1993) and grows on both south — and north-facing rocks. It is also found in more southerly regions, including subtropical Okinawa Island, where it grows on rocks in low light conditions beneath overhangs in the upper intertidal zone (Masuda & Kamura, unpublished observations). By contrast, I. calliclada grows on south-facing rocks or corallinaceous algae in the lower intertidal zone on tropical Phu Quae Island, the only locality in which it has been collected. These observations suggest.

that the two species have different ecological preferences.

A Hawaiian species, *L. crustiformans* McDermid, has longitudinally oriented secondary pit-connection between contiguous superficial cortical cells. However, it has a spreading basid disc and perpendicularly arranged tetrasporangia (McDermid, 1989), thus differing from *L. calliclada*.

Some Pacific American species with a single attachment disc deserve discussion. Laurentic asterbanisma Setchell et Gardner and L. subdisticha Dawson, Pusshul et Wildman differ from L. cullicidad in having compressed axes (Setchell & Gardner, 1924; Dawson et al., 1960). Laurencla subcorymbosa Dawson is distinguished from L. cullicidad by the presence of lenticular thickenings and deciduous branchlets that function as propagules (Dawson, 1963).

Laurencia calliclada produces a characteristic triterpenoid, calliclado [Suzuki et al., 1995.) Many kinds of halogenated secondary metabolites have been reported from various species of Laurencia. These belong to four structural classes: sesquiterpenoid, diterpenoid, triterpenoid and C15 acetogenin (Erickson, 1983). Among halogenated triterpenoids, callicladol is unique in having a hydroxyl substituent at C-5 which has not yet been found in squalene-derived polyethers isolated from other species of Laurencia (Suzuki et al., 1995, and references cited thereim).

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